Search Strategy

FILE 'USPATFULL' ENTERED AT 15:30:18 ON 28 SEP 2003

		E	KANG CHIL YONG/IN
L1	2	S	E3
		E	LI YAN/IN
L2	15	S	E3
L3	15	S	L2 NOT L1
L4	27728	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5	5431	S	L4 AND (SIGNAL SEQUENCE)
L6	1242	S	L5 AND (ENVELOPE)
L7	741	S	L6 AND (CYTOTOXIC OR CYTOLYTIC)
L8	44	S	L7 AND (SIGNAL SEQUENCE/CLM)
L9	44	S	L8 AND (ENV? OR GP160 OR GP120 OR GP41)
L10	18	S	L9 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM)
L11	741	S	L7 AND (ENV? OR GP160 OR GP120 OR GP41)
L12	82	S	L11 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM)
L13	57	S	L12 AND SOLUBLE
L14	49	S	L13 NOT L10
		E	S L6 AND SOLUBLE/CLM
L15	102	S	L6 AND SOLUBLE/CLM
L16	16	S	L15 AND (GP160/CLM OR GP120/CLM OR GP41/CLM OR RGP160/CLM OR
L17	16	S	L16 NOT L10

FILE 'MEDLINE' ENTERED AT 15:46:35 ON 28 SEP 2003

		E	KANG C Y/AU
		E	KANG CHIL YONG/AU
L18	1	S	E3
		E	LI YAN/AU
L19	127	S	E3
L20	1	S	L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21	134558	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L22	42	S	L21 AND (SIGNAL SEQUENCE)
L23	4684	S	L21 AND (CYTOLYTIC OR CYTOTOXIC OR SYNCYTI?)
L24	309	S	L23 AND CYTOLYTIC
L25	102	S	L24 AND (ENV? OR GP160 OR GP120 OR GP41)
L26	1615	S	L23 AND (SYNCYTI?)
L27	788	S	L26 AND (ENV? OR GP160 OR GP120 OR GP41)
L28	37	S	L27 AND (CELL DEATH)
L29	612	S	L21 AND (SI OR NSI OR SYNCYTIUM-INDUCING OR NON-SYNCYTIUM-IND
L30	206	S	L29 AND (ENV? OR GP160 OR GP120 OR GP41)
L31	104	S	L30 AND (SIGNAL? OR DETERMINANT? OR REGION?)
L32	103	S	L31 NOT L28
L33	103	S	L32 NOT L22
L34	2	S	L33 AND SIGNAL
L35	101	S	L33 NOT L34

L1 ANSWER 1 OF 2 USPATFULL on STN
96:111363 Chimeric immunogenic gag-V3 virus-like particles of the human immunodeficiency virus (HIV).
Kang, Chil-Yong, London, Canada
Luo, Lizhong, London, Canada
Korea Green Cross Corporation, Kyongki-Do, Korea, Republic of (non-U.S. corporation)
US 5580773 19961203
APPLICATION: US 1993-100118 19930730 (8)
PRIORITY: KR 1992-10493 19920617
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An unprocessed human immunodeficiency virus 2 (HIV-2) gag precursor protein, containing a deficient protease, assembles into virus-like particles by budding through the cytoplasmic domain of baculovirus-infected cells. Chimeric constructs were generated by coupling the truncated HIV-2 gag gene to the neutralizing domain (V3) or the neutralizing and CD4 binding domains (V3+CD4B) of gp120 env gene sequences obtained from HIV-1 or HIV-2. Virus-like particles were formed by chimeric gene products when the env gene sequences were linked to the 3' terminus of the gag gene. The gag-env chimeric proteins displayed immunoreactivity towards anti-gp120 rabbit antisera.

CLM What is claimed is:
1. A recombinant, chimeric, immunogenic gag-env virus-like particle of human immunodeficiency virus (HIV), comprising: (i) an HIV-2 Gag protein which extends from the amino terminus of Gag to a minimum of amino acid 376 and a maximum of amino acid 425, such that said Gag protein is capable of forming virus-like particles; and, (ii) an HIV Env protein linked to the C-terminus of Gag containing at least one virus-neutralizing epitope.

2. The recombinant, chimeric, immunogenic HIV gag-env virus-like particle according to claim 1, wherein the gag coding region includes at least one proline residue at amino acid positions 373, 375, or 377.

3. The recombinant, chimeric, immunogenic HIV gag-env virus-like particle according to claim 1, wherein the env gene encodes for a 198 amino acid segment containing the HIV-2 V3 loop and CD4 binding domain, and the gag-env coding region comprises 574-623 amino acids.

4. The recombinant, chimeric, immunogenic HIV gag-env virus-like particle according to claim 1, wherein the env gene encodes for the V3 loop of HIV gp120, the CD4-binding domain of HIV gp120, or a combination thereof.

5. The recombinant, chimeric, immunogenic HIV gag-env virus-like particle according to claim 4, wherein the env gene encodes for a 91 amino acid segment containing the HIV-1 gp120 V3 loop; a 90 amino acid segment containing the HIV-2 gp120 V3 loop; or a 198 amino acid segment containing the HIV-2 V3 loop and CD4-binding domain.

6. The recombinant, chimeric, immunogenic HIV gag-env virus-like particle according to claim 1, wherein the env gene encodes for at least two HIV-1 gp120 V3 loops placed in tandem.

7. The recombinant, chimeric, immunogenic HIV gag-env virus-like particle according to claim 6, wherein at least two of the HIV-1 gp120 V3 loops correspond to different viral isolates.

8. A method for the detection of antibodies directed against human immunodeficiency virus (HIV) in human biological specimens, comprising: (i) immobilizing the recombinant, chimeric, immunogenic HIV gag-env virus-like particle of claim 1 on a solid support; (ii) contacting said recombinant particle with a human biological specimen and allowing immobilized antigen-antibody complex formation to occur; (iii) washing away unbound antibodies and antigens from the immune complexes of step (ii); and (iv) detecting said immune complexes by the addition of a second labeled anti-human antibody.

L1 ANSWER 2 OF 2 USPATFULL on STN
95:75739 Chimeric HIV-2 gag particles.
Kang, Chil-Yong, London, Canada
Luo, Lizhong, London, Canada
Korea Green Cross Corporation, Korea, Republic of (non-U.S. corporation) a part interest
US 5443828 19950822
APPLICATION: US 1992-992618 19921218 (7)
PRIORITY: KR 1992-10493 19920617
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The chimeric proteins, and a protential vaccine and diagnostic reagent comprising gag-env chimeric protein particles are disclosed. The preparation comprises linking gag of HIV-2 to env to form the chimeric gene, inserting the obtained chimeric gene into the DNA of a baculovirus, infecting insect cells or insect host with the resulting recombinant virus, culturing it and purifying the obtained chimeric protein. The gag chimeric protein of HIV according to the present invention retains both antigenic and immunogenic properties.

CLM What is claimed is:
1. A recombinant Gag-Env chimeric polypeptide particle of human immunodeficiency virus, comprising: an HIV-2 Gag polypeptide selected from the group consisting of HIV-2 Gag polypeptides which extend from the N-terminal amino acid of Gag to a minimum of amino acid 376 and a maximum of amino acid 425 and wherein said Gag polypeptides have a proline at amino acid 373, 375 or 377; and an Env polypeptide from HIV comprising the V3 loop domain of gp120; the Env polypeptide linked to the C-terminus of the Gag polypeptide.

2. The Gag-Env chimeric polypeptide particle according to claim 1, wherein the Env polypeptide comprises the V3 loop domain of gp120 and the CD4 binding site.

3. The Gag-Env chimeric polypeptide particle according to claim 1, wherein the Gag-Env chimeric polypeptide is expressed in an insect cell by a recombinant baculovirus comprising a chimeric gag-env gene encoding the Gag-Env chimeric polypeptide.

4. The Gag-Env chimeric polypeptide particle according to claim 3, wherein the recombinant baculovirus is ATCC deposit number VR2316 or VR2317.

L10 ANSWER 12 OF 18 USPATFULL on STN
2000:91547 HIV envelope polypeptides and vaccine.
Berman, Phillip W., Portola Valley, CA, United States
Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)
US 6090392 20000718

APPLICATION: US 1997-889841 19970708 (8)
PRIORITY: US 1996-676737P 19960708 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Oligonucleotide sequences encoding gp120 polypeptides from breakthrough isolates of vaccine trials using MN-rgp120 and the encoded gp120 polypeptides are provided. Use of the gp120 polypeptides from one or more of the isolates in a subunit vaccine, usually together with MN-rgp120, can provide protection against HIV strains that are sufficiently different from the vaccine strain (e.g.; MN-rgp120) that the vaccine does not confer protection against those strains. Antibodies induced by the polypeptides are also provided.

CLM What is claimed is:

1. An isolated polypeptide comprising an HIV gp 120 amino acid sequence selected from the group consisting of Sequence ID Nos: 2, 5, 8, 10, 12, 16, 19, 23, 25, 28, 31, 33, 36, and 39, and fragments thereof, wherein each of said fragments comprises at least the V2, V3, and C4 domains of gp120.

2. The polypeptide of claim 1 wherein the polypeptide additionally comprises a flag epitope sequence.

3. The polypeptide of claim 2 wherein the flag epitope sequence is the HSV gD-1 flag epitope sequence.

4. The polypeptide of claim 2 wherein the flag epitope sequence is fused to the HIV gp120 amino acid sequence.

5. The polypeptide of claim 1 wherein said HIV gp 120 amino acid sequence is a fragment lacking the gp120 signal sequence.

6. The polypeptide of claim 5 wherein the polypeptide additionally comprises a flag epitope sequence.

7. The polypeptide of claim 5 wherein the flag epitope sequence is the HSV gD-1 flag epitope sequence.

8. The polypeptide of claim 1 wherein the polypeptide comprises an amino acid sequence selected from the group consisting of Sequence ID Nos. 2 and 5 and fragments thereof, wherein each of said fragments comprises at least the V2, V3, and C4 domains of gp120.

9. The polypeptide of claim 1 wherein the polypeptide comprises an amino acid sequence selected from the group consisting of Sequence ID Nos. 8 and 10 and fragments thereof, wherein each of said fragments comprises at least the V2, V3, and C4 domains of gp120.

10. The polypeptide of claim 1 wherein the polypeptide comprises an amino acid sequence selected from the group consisting of Sequence ID Nos. 12 and 16 and fragments thereof, wherein each of said fragments comprises at least the V2, V3, and C4 domains of gp120.

11. The polypeptide of claim 1 wherein the polypeptide comprises an amino acid sequence selected from the group consisting of Sequence ID Nos. 19 and 23 and fragments thereof, wherein each of said fragments comprises at least the V2, V3, and C4 domains of gp120.

12. The polypeptide of claim 1 wherein the polypeptide comprises an amino acid sequence selected from the group consisting of Sequence ID Nos. 25 and 28 and fragments thereof, wherein each of said fragments comprises at least the V2, V3, and C4 domains of gp120.

13. The polypeptide of claim 1 wherein the polypeptide comprises an amino acid sequence selected from the group consisting of Sequence ID Nos. 31 and 33 and fragments thereof, wherein each of said fragments comprises at least the V2, V3, and C4 domains of gp120.

14. The polypeptide of claim 1 wherein the polypeptide comprises an amino acid sequence selected from the group consisting of Sequence ID Nos. 36 and 39 and fragments thereof, wherein each of said fragments comprises at least the V2, V3, and C4 domains of gp120.

15. An oligonucleotide of not more than five kilobases encoding an HIV gp120 polypeptide sequence comprising an amino acid sequence selected from the group consisting of Sequence ID Nos. 2, 5, 8, 10, 12, 16, 19, 23, 25, 28, 31, 33, 36, and 39, and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120.

16. The oligonucleotide of claim 15 wherein the oligonucleotide additionally encodes a flag epitope.

17. The oligonucleotide of claim 15 wherein the flag epitope is the HSV gD-1 flag epitope.

18. The oligonucleotide of claim 16 wherein the sequence encoding the flag epitope is fused to the sequence encoding the HIV gp120 amino acid sequence.

19. The oligonucleotide of claim 15 wherein said amino acid sequence is a fragment lacking the gp120 signal sequence
.

20. The oligonucleotide of claim 19 wherein the nucleotide sequence encoding said fragment is joined to a nucleotide sequence encoding a heterologous signal sequence.

21. The oligonucleotide of claim 20 wherein said nucleotide sequences are joined via a nucleotide sequence encoding a flag epitope sequence.

22. The oligonucleotide of claim 20 wherein the signal sequence is the HSV gD1 signal sequence and the flag epitope sequence is the HSV gD-1 flag epitope sequence.

23. A vector comprising the oligonucleotide of claim 15.

24. A host cell comprising the vector of claim 23.

25. A method of producing a polypeptide comprising culturing the host cell of claim 24 and recovering the polypeptide.

26. An oligonucleotide of not more than 5 kilobases comprising a nucleotide sequence selected from the group consisting of Sequence ID Nos: 1, 4, 7, 9, 11, 15, 18, 22, 24, 27, 30, 32, 35, and 38, and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120.

27. The oligonucleotide of claim 26 wherein the oligonucleotide

comprises a nucleotide sequence selected from the group consisting of Sequence ID Nos. 1 and 4 and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120
.

28. The oligonucleotide of claim 26 wherein the oligonucleotide comprises a nucleotide sequence selected from the group consisting of Sequence ID Nos. 7 and 9 and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120
.

29. The oligonucleotide of claim 26 wherein the oligonucleotide comprises a nucleotide sequence selected from the group consisting of Sequence ID Nos. 11 and 15 and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120
.

30. The oligonucleotide of claim 26 wherein the oligonucleotide comprises a nucleotide sequence selected from the group consisting of Sequence ID Nos. 18 and 22 and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120
.

31. The oligonucleotide of claim 26 wherein the oligonucleotide comprises a nucleotide sequence selected from the group consisting of Sequence ID Nos. 24 and 27 and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120
.

32. The oligonucleotide of claim 26 wherein the oligonucleotide comprises a nucleotide sequence selected from the group consisting of Sequence ID Nos. 30 and 32 and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120
.

33. The oligonucleotide of claim 26 wherein the oligonucleotide comprises a nucleotide sequence selected from the group consisting of Sequence ID Nos. 35 and 38 and fragments thereof, wherein each of said fragments encodes at least the V2, V3, and C4 domains of gp120
.

L17 ANSWER 16 OF 16 USPATFULL on STN
92:100919 Viral vector coding glycoprotein of HIV-1.
Kieny, Marie-Paule, Strasbourg, France
Rautmann, Guy, Strasbourg, France
Lecocq, Jean-Pierre, Reichstett, France
Hobson, Simon W., Montigny-le-Bretonneux, France
Girard, Marc, Paris, France
Montagnier, Luc, Le Plessis-Robinson, France
Transgene S.A., Institut Pasteur, France (non-U.S. corporation)
US 5169763 19921208
APPLICATION: US 1991-765413 19910924 (7)
PRIORITY: FR 1986-5043 19860408
FR 1986-15106 19861029
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A viral vector comprising at least a portion of the genome of the HIV virus, a gene coding gp160 glycoprotein of the envelope of the HIV virus, as well as the elements providing for the expression of the glycoprotein in cells, wherein the

gp160 is expressed as a non-cleavable protein.

CLM What is claimed is:

1. A viral vector, the genome of which comprises: a functional origin of replication of a poxvirus; a first DNA fragment encoding a non-cleavable gp160, consisting of gp120-gp140, derived from the natural gp160 of an HIV-1 virus, said non-cleavable gp160 being characterized in that it does not contain the amino acid sequence REKR originally found in the natural gp160; a second DNA fragment encoding a signal peptide, said second DNA fragment being linked to the 5' end of said first DNA fragment; and a promoter for expressing said DNA fragment in mammalian cells.

2. A viral vector according to claim 1, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it does not contain the amino acid sequences KRR and REKR originally found in the natural gp160.

3. A viral vector according to claim 1, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR originally found in the natural gp160.

4. A viral vector according to claim 2, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR originally found in the natural gp160, and in that it comprises a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR originally found in the natural gp160.

5. A viral vector according to claim 3, the genome of which comprises a DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being different from the natural gp160 in that the amino acid sequence REKR originally found in the natural gp160 is replaced by the amino acid sequence NEHQ.

6. A viral vector according to claim 4, the genome of which comprises a DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being different from the natural gp160 in that the amino acid sequences KRR and REKR are replaced respectively by the amino acid sequences QNH and NEHQ.

7. A viral vector according to claim 1, the genome of which comprises a first DNA fragment encoding a non-cleavable and soluble gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable and soluble gp160 being different from the natural gp160 in that it does not contain the amino acid sequence REKR originally found in the natural gp160, and in that the transmembrane region originally found in the natural gp160 is deleted.

8. A viral vector according to claim 7, wherein said non-cleavable and soluble gp160 is different from the natural

gp160 in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR originally found in the natural gp160.

9. A viral vector according to claim 8, wherein said non-cleavable and soluble gp160 is different from the natural gp160 in that the amino acid sequence REKR originally found in the natural gp160 is replaced by the amino acid sequence NEHQ.

10. A viral vector according to claim 2, the genome of which comprises a first DNA fragment encoding a non-cleavable and soluble gp160 derived form the natural gp160 of the HIV-1 virus, said non-cleavable and soluble gp160 being different from the natural gp160 in that it does not contain the amino acid sequences KRR and REKR originally found in the natural gp160, and in that the transmembrane region originally found in the natural gp160 is deleted.

11. A viral vector according to claim 10, wherein said non-cleavable and soluble gp160 is different from the natural gp160 in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR originally found in the natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR originally found in the natural gp160.

12. A viral vector according to claim 11, wherein said non-cleavable and soluble gp160 is different from the natural gp160 in that the amino acid sequences KRR and REKR are replaced respectively by the amino acid sequences QNH and NEHQ.

13. A viral vector according to claim 1, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived form the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it does not contain the amino acid sequence REKR originally found in the natural gp160, and in that the transmembrane region originally found in the natural gp160 is replaced by the transmembrane region of the glycoprotein of the rabies virus.

14. A viral vector according to claim 13, wherein said non-cleavable gp160 is characterized in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR originally found in the natural gp160.

15. A viral vector according to claim 2, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it does not contain the amino acid sequences KRR and REKR originally found in the natural gp160, and in that the transmembrane region originally found in the natural gp160 is replaced by the transmembrane region of the glycoprotein of the rabies virus.

16. A viral vector according to claim 15, wherein said non-cleavable gp160 is characterized in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR originally found in the natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR originally found in the natural gp160.

17. A viral vector according to claim 1, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it does not contain the amino acid sequence REKR originally found in the natural gp160, and in that the amino acid Arg of the transmembrane region originally found in the natural gp160 is replaced by the amino acid Ile.

18. A viral vector according to claim 17, wherein said non-cleavable gp160 is characterized in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR originally found in the natural gp160.

19. A viral vector according to claim 2, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it does not contain the amino acid sequences KRR and REKR originally found in the natural gp160, and in that the amino acid Arg of the transmembrane region originally found in the natural gp160 is replaced by the amino acid Ile.

20. A viral vector according to claim 19, wherein said non-cleavable gp160 is characterized in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR originally found in the natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR originally found in the natural gp160.

21. A viral vector according to claim 1, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it does not contain the amino acid sequence REKR originally found in the natural gp160, and in that the hydrophobic region proximate to the C-terminal end of the REKR sequence as originally found in the natural gp160 is deleted.

22. A viral vector according to claim 2, the genome of which comprises a first DNA fragment encoding a non-cleavable gp160 derived from the natural gp160 of the HIV-1 virus, said non-cleavable gp160 being characterized in that it does not contain the amino acid sequences REKR and KRR originally found in the natural gp160, and in that the hydrophobic region proximate to the C-terminal end of the REKR sequence as originally found in the natural gp160 is deleted.

23. A viral vector according to claim 1, the genome of which comprises a second DNA fragment encoding a signal peptide which is selected from the group consisting of the signal peptide of the precursor of the gp160 of the HIV-1 virus and the signal peptide of the precursor of the glycoprotein of the rabies virus.

24. A viral vector according to claim 2, the genome of which comprises a second DNA fragment encoding a signal peptide which is selected from the group consisting of the signal peptide of the precursor of the gp160 of the HIV-1 virus and the signal peptide of the precursor of the glycoprotein of the rabies virus.

25. A viral vector according to claim 1, the genome of which comprises a

functional origin of replication of a poxvirus.

26. A viral vector according to claim 25, the genome of which comprises a functional origin of replication of a vaccinia virus.

27. A viral vector according to claim 2, the genome of which comprises a functional origin of replication of a poxvirus.

28. A viral vector according to claim 27, the genome of which comprises a functional origin of replication of a vaccinia virus.

29. A viral vector according to claim 1, wherein the DNA encoding envelope protein of HIV-1 is encoded by the EcoRI-KpnI and KpnI-HindIII fragments of plasmid PJ19-13, comprising nucleotides 1258 to 1698 of the DNA encoding envelope protein of HIV-1, and the HindIII-XhoI fragment of plasmid PJ19-6, comprising nucleotides 1698 to 9173 of the DNA encoding envelope protein of HIV-1.

30. A viral vector according to claim 2, wherein the DNA encoding envelope protein of HIV-1 is encoded by the EcoRI-KpnI and KpnI-HindIII fragments of plasmid PJ19-13, comprising nucleotides 1258 to 1698 of the DNA encoding envelope protein of HIV-1, and the HindIII-XhoI fragment of plasmid PJ19-6, comprising nucleotides 1698 to 9173 of the DNA encoding envelope protein of HIV-1.

31. A culture of mammalian cells, which is infected with a viral vector as claimed in any one of claims 1 to 6 or 13 to 28.

32. A culture of mammalian cells, which is infected with a viral vector as claimed in any one of claims 7 to 12.

33. A process for producing a non-cleavable and soluble gp160 which comprises recovering said non-cleavable gp160 from a culture of mammalian cells as claimed in claim 32.

L17 ANSWER 12 OF 16 USPATFULL on STN
97:89064 Non-cleavable GP160 glycoproteins of HIV.
Kieny, Marie-Paule, Strasbourg, France
Rautmann, Guy, Strasbourg, France
Lecocq, Jean-Pierre, Reichstett, France
Hobson, Simon Wain, Montigny-le-Bretonneux, France
Girard, Marc, Paris, France
Montagnier, Luc, Le Plessis-Robinson, France
Transgene S.A. and Institute Pasteur, Paris, France (non-U.S. corporation)
US 5672689 19970930
APPLICATION: US 1995-442995 19950517 (8)
PRIORITY: FR 1986-5043 19860408
FR 1986-15106 19861029
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A viral vector comprising at least a portion of the genome of the HIV virus, a gene encoding gp160 glycoprotein of the envelope of the HIV virus, as well as the elements providing for the expression of the glycoprotein in cells, wherein the gp160 is expressed as a non-cleavable protein.

CLM What is claimed is:

1. A non-cleavable gp160 glycoprotein, consisting essentially of gp120-gp40 of a human immunodeficiency virus Type 1 (HIV-1), wherein said gp160 does not contain the amino acid sequence REKR found in natural gp160, and wherein the transmembrane region found in natural gp160 is replaced by the transmembrane region of the glycoprotein of the rabies virus.

2. The gp160 according to claim 1, wherein said gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

3. A non-cleavable gp160 glycoprotein, consisting essentially of gp120-gp40 of a human immunodeficiency virus Type 1 (HIV-1), wherein said gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160, and wherein the transmembrane region found in natural gp160 is replaced by the transmembrane region of the glycoprotein of the rabies virus.

4. The gp160 according to claim 3, wherein said gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

5. A non-cleavable gp160 glycoprotein, consisting essentially of gp120-gp40 of a human immunodeficiency virus Type 1 (HIV-1), wherein said gp160 does not contain an amino acid sequence REKR found in natural gp160, further comprising a signal peptide selected from the group consisting of the signal peptide of the precursor of the gp160 of a HIV-1 virus and the signal peptide of the precursor of the glycoprotein of the rabies virus.

6. A non-cleavable gp160 glycoprotein, consisting essentially of gp120-gp40 of a human immunodeficiency virus Type 1 (HIV-1), wherein said gp160 does not contain an amino acid sequence REKR found in natural gp160, wherein the DNA encoding envelope protein of HIV-1 is encoded by the EcoRI-KpnKI and KpnI-HindIII fragments of plasmid PJ19-13, comprising nucleotides 1258 to 1698 of the DNA encoding envelope protein of HIV-1, and the HindIII-XhoI fragment of plasmid PJ19-6, comprising nucleotides 1698 to 9173 of the DNA encoding envelope protein of HIV-1.

7. A non-cleavable gp160 glycoprotein, consisting eassentially of gp120-gp40 of a human immunodeficiency virus Type 1 (HIV-1), wherein said gp160 does not contain an amino acid sequence REKR found in natural gp160, wherein said gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160, and wherein the DNA encoding envelope protein of HIV-1 envelope DNA is encoded by the EcoRI-KpnI and KpnI-HindIII fragments of the plasmid PJ19-13, comprising nucleotides 1258 to 1698 of the DNA encoding envelope protein of HIV-1, and the HindIII-XhoI fragment of plasmid PJ19-6, comprising nucletides 1698 to 9173 of the DNA encoding envelope protein of HIV-1.

8. A non-cleavable gp160 glycoprotein, consisting essentially of gp120-gp40 of a human immunodeficiency

virus Type 1 (HIV-1), wherein said gp160 does not contain an amino acid sequence REKR found in natural gp160.

9. The gp160 according to claim 8, wherein said gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160.

10. The gp160 according to claim 8, wherein said gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

11. The gp160 according to claim 9, wherein said gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

12. The gp160 according to claim 10, wherein the amino acid sequence REKR found in natural gp160 is replaced by the amino acid sequence NEHQ.

13. The gp160 according to claim 11, wherein the amino acid sequences KRR and REKR found in natural gp160 are replaced, respectively, by the amino acid sequences QNH and NEHQ.

14. A non-cleavable and soluble gp160 glycoprotein according to claim 8, wherein said gp160 does not contain the amino acid sequence REKR found in natural gp160, and said gp160 does not contain a transmembrane region.

15. The gp160 according to claim 14, wherein said gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

16. A non-cleavable and soluble gp160 glycoprotein according to claim 9, wherein said gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160, and said gp160 does not contain a transmembrane region.

17. The gp160 according to claim 16, wherein said gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR found in natural gp160.

18. A non-cleavable and soluble gp160 glycoprotein according to claim 8, wherein said gp160 does not contain the amino acid sequence REKR found in natural gp160, and the transmembrane region found in natural gp160 is deleted.

19. The gp160 according to claim 18, wherein said gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

20. The gp160 according to claim 19, wherein type amino acid sequence REKR found in natural gp160 is replaced by the amino acid sequence NEHQ.

21. A non-cleavable and soluble gp160 glycoprotein according to claim 9, wherein said gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160, and

the transmembrane region found in natural gp160 is deleted.

22. The gp160 according to claim 21, wherein said gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

23. The gp160 according to claim 22, wherein the amino acid sequences KRR and REKR found in natural gp160 are replaced, respectively, by the amino acid sequences QNH and NEHQ.

L17 ANSWER 11 OF 16 USPATFULL on STN
1998:98607 Viral vector coding for a glycoprotein of the virus responsible for A.I.D.S..
Kieny, Marie-Paule, Strasbourg, France
Rautmann, Guy, Strasbourg, France
Lecocq, Jean-Pierre, Reichstett, France
Hobson, Simon Wain, Montigny-le-Bretonneux, France
Girard, Marc, Paris, France
Montagnier, Luc, Le Plessis-Robinson, France
Transgene S.A., Courbevoie, France (non-U.S. corporation)Institut Pasteur, Paris, France (non-U.S. corporation)
US 5795577 19980818
APPLICATION: US 1995-481622 19950607 (8)
PRIORITY: FR 1986-5043 19860408
FR 1986-15106 19861029
WO 1987-FR116 19870408
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to an immunogenic composition comprising a viral vector. The genome of the viral vector comprises a functional origin of replication of a poxvirus, a DNA fragment encoding a non-cleavable gp160, a DNA fragment encoding a signal peptide, and a promoter for expressing DNA fragments in mammalian cells.

CLM What is claimed is:
1. An immunogenic composition comprising a viral vector, the genome of which comprises: a functional origin of replication of a poxvirus; a first DNA fragment encoding a non-cleavable gp160, consisting of gp120-gp40 of an HIV-1 virus, wherein said non-cleavable gp160 does not contain the amino acid sequence REKR found in natural gp160; a second DNA fragment encoding a signal peptide, said second DNA fragment being linked to the 5'end of said first DNA fragment; and a promoter for expressing said DNA fragments in mammalian cells.

2. The immunogenic composition of claim 1, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp160 of the HIV-1 virus, wherein said non-cleavable gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160.

3. The immunogenic composition of claim 1, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp160 of the HIV-1 virus, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

4. The immunogenic composition of claim 2, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp160 of HIV-1 virus, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and in that said non-cleavable gp160 comprises a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

5. The immunogenic composition of claim 3, wherein said genome of said viral vector comprises a DNA fragment encoding a non-cleavable gp160 of HIV-1 virus, wherein said non-cleavable gp160 is different from natural gp160 in that the amino acid sequence REKR found in the natural gp160 is replaced by the amino acid sequence NEHQ.

6. The immunogenic composition of claim 4, wherein said genome of said viral vector comprises a DNA fragment encoding a non-cleavable gp160 of HIV-1 virus, wherein said non-cleavable gp160 is different from natural gp160 in that the amino acid sequences KRR and REKR are replaced, respectively, by the amino acid sequences QNH and NEHQ.

7. The immunogenic composition of claim 1, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable and soluble gp160 of HIV-1 virus, wherein said non-cleavable and soluble gp160 is different from natural gp160 in that it does not contain the amino acid sequence REKR found in natural gp160, and in that the transmembrane region found in natural gp160 is deleted.

8. The immunogenic composition of claim 7, wherein said non-cleavable and soluble gp160 is different from natural gp160 in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

9. The immunogenic composition of claim 8, wherein said non-cleavable and soluble gp160 is different from natural gp160 in that the amino acid sequence REKR found in natural gp160 is replaced by the amino acid sequence NEHQ.

10. The immunogenic composition of claim 2, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable and soluble gp160 of HIV-1 virus, wherein said non-cleavable and soluble gp 160 is different from natural gp 160 in that it does not contain the amino acid sequences KRR and REKR found in natural gp160, and in that the transmembrane region found in natural gp160 is deleted.

11. The immunogenic composition of claim 10, wherein said non-cleavable and soluble gp160 is different from natural gp160 in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

12. The immunogenic composition of claim 11, wherein said non-cleavable and soluble gp160 is different from natural gp160 in that the amino acid sequences KRR and REKR are replaced, respectively, by the amino acid sequences QNH and NEHQ.

13. The immunogenic composition of claim 1, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp 160 of HIV-1 virus, wherein said non-cleavable gp160 does not contain the amino acid sequence REKR found in natural gp160, and in that the transmembrane region found in natural gp160 is replaced by the transmembrane region of the glycoprotein of the rabies virus.

14. The immunogenic composition of claim 13, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp166.

15. The immunogenic composition of claim 2, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp160 of HIV-1 virus, wherein said non-cleavable gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160, and in that the transmembrane region found in natural gp160 is replaced by the transmembrane region of the glycoprotein of the rabies virus.

16. The immunogenic composition of claim 15, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

17. The immunogenic composition of claim 1, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp160 of HIV-1 virus, wherein said non-cleavable gp160 does not contain the amino acid sequence REKR found in natural gp160, and in that the amino acid Arg of the transmembrane region found in natural gp160 is replaced by the amino acid Ile.

18. The immunogenic composition of claim 17, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

19. The immunogenic composition of claim 2, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp160 of HIV-1 virus, wherein said non-cleavable gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160, and in that the amino acid Arg of the transmembrane region found in natural gp160 is replaced by the amino acid Ile.

20. The immunogenic composition of claim 19, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

21. The immunogenic composition of claim 1, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp160 of HIV-1 virus, wherein said non-cleavable gp160 does not contain the amino acid sequence REKR found in natural gp160, and in that the hydrophobic region proximate the C-terminal end of the REKR sequence as found in natural gp160 is deleted.

22. The immunogenic composition of claim 2, wherein said genome of said viral vector comprises a first DNA fragment encoding a non-cleavable gp160 of HIV-1 virus, said non-cleavable gp160 does not contain the amino acid sequences REKR and KRR found in natural gp160, and in that the hydrophobic region proximate to the C-terminal end of the REKR sequence found in natural gp160 is deleted.

23. The immunogenic composition of claim 1, wherein said genome of said viral vector comprises a second DNA fragment encoding a signal peptide selected from the group consisting of the signal peptide of the precursor of gp160 of HIV-1 and the signal peptide of the precursor of the glycoprotein of rabies virus.

24. The immunogenic composition of claim 2, wherein said genome of said viral vector comprises a second DNA fragment encoding a signal peptide selected from the group consisting of the signal peptide of the precursor of gp160 of HIV-1 and the signal peptide of the precursor of the glycoprotein of rabies virus.

25. The immunogenic composition of claim 1, wherein said genome of said viral vector comprises a functional origin of replication of a vaccinia virus.

26. The immunogenic composition of claim 2, wherein said genome of said viral vector comprises a functional origin of replication of a vaccinia virus.

27. The immunogenic composition of claim 1, wherein the DNA encoding envelope protein of HIV-1 is comprised of EcoRI-KpnI and KpnI-HindIII fragments of plasmid PJ19-13, comprising nucleotides 1258 to 1698 of the DNA encoding envelope protein of HIV-1, and the HindlII-XhoI fragment of the plasmid PJ19-6, comprising nucleotides 1698 to 9173 of the DNA encoding envelope protein of HIV-1.

28. The immunogenic composition of claim 2, wherein the DNA encoding envelope protein of HIV-1 is comprised of EcoRI-KpnI and KpnI-HindIII fragments of plasmid PJ19-13, comprising nucleotides 1258-1698 of the DNA encoding envelope protein of HIV -1, and the HindIII-XhoI fragment of plasmid PJ19-6, comprising nucleotides 1698 to 9173 of the DNA encoding envelope protein of HIV-1.

29. An immunogenic composition comprising a non-cleavable gp160 glycoprotein, consisting essentially of gp120-gp40 of a human immunodeficiency virus Type 1 (HIV-1), wherein said gp160 does not contain the amino acid sequence REKR found in natural gp160, and a carrier.

30. The immunogenic composition of claim 29, wherein said non-cleavable gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160.

31. The immunogenic composition of claim 29, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

32. The immunogenic composition of claim 30, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and in

that it comprises a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

33. The immunogenic composition of claim 31, wherein said non-cleavable gp160 is different from natural gp160 in that the amino acid sequence REKR found in the natural gp160 is replaced by the amino acid sequence NEHQ.

34. The immunogenic composition of claim 32, wherein said non-cleavable gp160 is different from natural gp160 in that the amino acid sequences KRR and REKR are replaced, respectively, by the amino acid sequences QNH and NEHQ.

35. The immunogenic composition of claim 29, wherein said non-cleavable gp160 is soluble and different from natural gp160 in that it does not contain the amino acid sequence REKR found in natural gp160, and in that the transmembrane region found in natural gp160 is deleted.

36. The immunogenic composition of claim 35, wherein said non-cleavable and soluble gp160 is different from natural gp160 in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp 160.

37. The immunogenic composition of claim 36, wherein said non-cleavable and soluble gp160 is different from natural gp 160 in that the amino acid sequence REKR found in natural gp160 is replaced by the amino acid sequence NEHQ.

38. The immunogenic composition of claim 30, wherein said non-cleavable and soluble gp160 is soluble and different from natural gp160 in that it does not contain the amino acid sequences KRR and REKR found in natural gp160, and in that the transmembrane region found in natural gp160 is deleted.

39. The immunogenic composition of claim 38, wherein said non-cleavable and soluble gp160 is different from natural gp160 in that it comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

40. The immunogenic composition of claim 39, wherein said non-cleavable and soluble gp160 is different from natural gp 160 in that the amino acid sequences KRR and REKR are replaced, respectively, by the amino acid sequences QNH and NEHQ.

41. The immunogenic composition of claim 29, wherein said non-cleavable gp160 does not contain the amino acid sequence REKR found in natural gp160, and in that the transmembrane region found in natural gp160 is replaced by the transmembrane region of the glycoprotein of the rabies virus.

42. The immunogenic composition of claim 41, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

43. The immunogenic composition of claim 30, wherein said non-cleavable gp160 does not contain the amino acid sequences KRR and REKR found in natural gp160, and in that the transmembrane region found in natural gp 160 is replaced by the transmembrane region of the

glycoprotein of the rabies virus.

44. The immunogenic composition of claim 43, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

45. The immunogenic composition of claim 29, wherein said non-cleavable gp160 does not contain the amino acid sequence REKR found in natural gp160, and in that the amino acid Arg of the transmembrane region found in natural gp160 is replaced by the amino acid Ile.

46. The immunogenic composition of claim 45, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160.

47. The immunogenic composition of claim 30, wherein said non-cleavable gp160 does not contain the amino acid sequences KRR and REKR found in natural gp 160, and in that the amino acid Arg of the transmembrane region found in natural gp160 is replaced by the amino acid Ile.

48. The immunogenic composition of claim 47, wherein said non-cleavable gp160 comprises a 4-amino acid sequence other than REKR in place of the amino acid sequence REKR found in natural gp160, and a 3-amino acid sequence other than KRR in place of the amino acid sequence KRR found in natural gp160.

49. The immunogenic composition of claim 29, wherein said non-cleavable gp160 does not contain the amino acid sequence REKR found in natural gp160, and in that the hydrophobic region proximate the C-terminal end of the REKR sequence as found in natural gp160 is deleted.

50. The immunogenic composition of claim 30, wherein said non-cleavable gp160 does not contain the amino acid sequences REKR and KRR found in natural gp160, and in that the hydrophobic region proximate to the C-terminal end of the REKR sequence found in natural gp160 is deleted.

51. The immunogenic composition of claim 29, wherein said genome of said viral vector comprises a second DNA fragment encoding a signal peptide selected from the group consisting of the signal peptide of the precursor of gp160 of HIV-1 and the signal peptide of the precursor of the glycoprotein of rabies virus.

52. The immunogenic composition of claim 30, wherein said genome of said viral vector comprises a second DNA fragment encoding a signal peptide selected from the group consisting of the signal peptide of the precursor of gp160 of HIV-1 and the signal peptide of the precursor of the glycoprotein of rabies virus.

53. A purified antibody directed against the immunogenic composition according to any one of claims 1-52.

54. A method of producing an antibody comprising the steps of (a) immunizing a host animal with an immunogenic composition of any one of claims 1-52; and (b) isolating the antibodies from the sera of said host animal.

55. A purified antibody produced by the method of claim 54.

L17 ANSWER 7 OF 16 USPATFULL on STN
2001:112067 Expression cassettes encoding soluble, non-cleavable, chimeric HIV-1 GP160 variants, their methods of construction, and methods for the production of GP160 variants.
Kieny, Marie-Paule, Strasbourg, France
Transgene S.A., Strasbourg, France (non-U.S. corporation)
US 6261799 B1 20010717
WO 9219742 19921112
APPLICATION: US 1992-956483 19921231 (7)
WO 1992-FR394 19920430 19921231 PCT 371 date 19921231 PCT 102(e) date
PRIORITY: FR 1991-5392 19910502
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention is directed toward soluble, non-cleavable, chimeric human immunodeficiency virus type 1 (HIV-1) gp160 variants, expression vectors encoding said variants, and methods of producing said variants. These HIV-1 gp160 variants comprise the following regions: I) a first region derived from the gp160 of a first strain of HIV-1; ii) a second region derived from the gp160 of a second strain of HIV-1 wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, derived from the gp160 of said second strain, located at the amino terminus of the recombinant envelope.

CLM What is claimed is:
1. A method for constructing a human immunodeficiency virus type 1 (HIV-1) gp160 expression cassette, wherein said expression cassette contains a nucleic acid capable of encoding a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV-1 consisting of amino acids X to Y, wherein X is a number from 1 to 271 and Y is a number from 306 to 482; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said region derived from the gp160 of said second strain of HIV-1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU; said method comprising the following steps: i) cloning and isolating DNA fragments encoding said first, second, and third regions of the HIV-1 envelope; ii) inserting said DNA fragments into an appropriate site in an expression cassette, wherein said cassette comprises a promoter, translation initiation codon, and optional nucleic acid encoding a signal peptide in sequence and upstream of the insertion site, and a translation termination site downstream of the insertion site.

2. The method according to claim 1, wherein said expression cassette

contains a nucleic acid sequence encoding a signal peptide fused to the inserted DNA fragments encoding said first, second, and third regions of the HIV-1 envelope.

3. The method according to claim 1, wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

4. The method according to claim 1, wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

5. An expression cassette containing a nucleic acid capable of encoding a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV-1 consisting of amino acids X to Y, wherein X is a number from 1 to 271 and Y is a number from 306 to 482; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said region derived from the gp160 of said second strain of HIV -1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU; said expression cassette further comprising: i) a promoter, translation initiation codon, and optional nucleic acid encoding a signal peptide in sequence and upstream of the insertion site, and a translation termination site downstream of the insertion site.

6. The expression cassette according to claim 5, wherein said expression cassette contains a nucleic acid sequence encoding a signal peptide fused to the amino terminus of said first, second, and third regions of the HIV-1 envelope.

7. The expression cassette according to claim 5, wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

8. The expression cassette according to claim 5, wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

9. A method for constructing a human immunodeficiency virus type 1 (HIV-1) gp160 expression cassette, wherein said expression cassette contains a nucleic acid capable of encoding a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV-1 consisting of amino acids X to Y, wherein X is a number from 1 to 271 and Y is a number from 306 to 476; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said

region derived from the gp160 of said second strain of HIV-1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU; said method comprising the following steps: i) cloning and isolating DNA fragments encoding said first, second, and third regions of the HIV-1 envelope; ii) inserting said DNA fragments into an appropriate site in an expression cassette, wherein said cassette comprises a promoter, translation initiation codon, and optional nucleic acid encoding a signal peptide in sequence and upstream of the insertion site, and a translation termination site downstream of the insertion site.

10. The method according to claim 9, wherein said expression cassette contains a nucleic acid sequence encoding a signal peptide fused to the inserted DNA fragments encoding said first, second, and third regions of the HIV-1 envelope.

11. The method according to claim 9, wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

12. The method according to claim 9, wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

13. An expression cassette containing a nucleic acid capable of encoding a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV-1 consisting of amino acids X to Y, wherein S is a number from 1 to 271 and Y is a number from 306 to 476; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said region derived from the gp160 of said second strain of HIV -1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU; said expression cassette further comprising: i) a promoter, translation initiation codon, and optional nucleic acid encoding a signal peptide in sequence and upstream of the insertion site, and a translation termination site downstream of the insertion site.

14. The expression cassette according to claim 13, wherein said expression cassette contains a nucleic acid sequence encoding a signal peptide fused to the amino terminus of said first, second, and third regions of the HIV-1 envelope.

15. The expression cassette according to claim 13, wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

16. The expression cassette according to claim 13, wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

17. A method for constructing a human immunodeficiency virus type 1 (HIV-1) gp160 expression

cassette, wherein said expression cassette contains a nucleic acid capable of encoding a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV-1 consisting of amino acids X to Y, wherein X is a number from 1 to 271 and Y is a number from 450 to 482; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said region derived from the gp160 of said second strain of HIV-1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU; said method comprising the following steps: i) cloning and isolating DNA fragments encoding said first, second, and third regions of the HIV-1 envelope; ii) inserting said DNA fragments into an appropriate site in an expression cassette, wherein said cassette comprises a promoter, translation initiation codon, and optional nucleic acid encoding a signal peptide in sequence and upstream of the insertion site, and a translation termination site downstream of the insertion site.

18. The method of claim 17, wherein said expression cassette contains a nucleic acid sequence encoding a signal peptide fused to the inserted DNA fragments encoding said first, second, and third regions of the HIV-1 envelope.

19. The method according to claim 17, wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

20. The method according to claim 17, wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

21. An expression cassette containing a nucleic acid capable of encoding a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV-1 consisting of amino acids X to Y, wherein X is a number from 1 to 271 and Y is a number from 450 to 482; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said region derived from the gp160 of said second strain of HIV -1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU; said expression cassette further comprising: i) a promoter, translation initiation codon, and optional nucleic acid encoding a signal peptide in sequence and upstream of the insertion site, and a translation termination site downstream of the insertion site.

22. The expression cassette according to claim 21, wherein said expression cassette contains a nucleic acid sequence encoding a signal

peptide fused to the amino terminus of said first, second, and third regions of the HIV-1 envelope.

23. The expression cassette according to claim 21, wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

24. The expression cassette according to claim 21, wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

25. A method for constructing a human immunodeficiency virus type 1 (HIV-1) gp160 expression cassette, wherein said expression cassette contains a nucleic acid capable of encoding a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV-1 consisting of amino acids X to Y, wherein X is a number from 1 to 97 and Y is a number from 306 to 482; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said region derived from the gp160 of said second strain of HIV-1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU; said method comprising the following steps: i) cloning and isolating DNA fragments encoding said first, second, and third regions of the HIV-1 envelope; ii) inserting said DNA fragments into an appropriate site in an expression cassette, wherein said cassette comprises a promoter, translation initiation codon, and optional nucleic acid encoding a signal peptide in sequence and upstream of the insertion site, and a translation termination site downstream of the insertion site.

26. The method according to claim 25, wherein said expression cassette contains a nucleic acid sequence encoding a signal peptide fused to the inserted DNA fragments encoding said first, second, and third regions of the HIV-1 envelope.

27. The method according to claim 25, wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

28. The method according to claim 25, wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

29. An expression cassette containing a nucleic acid capable of encoding a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV-1 consisting of amino acids X to Y, wherein X is a number from 1 to 97 and Y is a number from 306 to 482; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and

475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said region derived from the gp160 of said second strain of HIV -1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU; said expression cassette further comprising: i) a promoter, translation initiation codon, and optional nucleic acid encoding a signal peptide in sequence and upstream of the insertion site, and a translation termination site downstream of the insertion site.

30. The expression cassette according to claim 29, wherein said expression cassette contains a nucleic acid sequence encoding a signal peptide fused to the amino terminus of said first, second, and third regions of the HIV-1 envelope.

31. The expression cassette according to claim 29, wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

32. The expression cassette according to claim 29, wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

33. A viral vector which contains an expression cassette according to claim 5.

34. A cell transfected with a viral vector according to claim 33.

35. A method of using the cell of claim 34 to produce a soluble , non-cleavable, chimeric HIV-1 gp160 variant comprising culturing said cell under conditions that provide for expression of said variant, and harvesting said variant from the cell culture.

36. A viral vector which contains an expression cassette according to claim 21.

37. A cell transfected with a viral vector according to claim 36.

38. A method of using the cell of claim 37 to produce a soluble , non-clcavable, chimeric HIV-1 gp160 variant comprising culturing said cell under conditions that provide for expression of said variant, and harvesting said variant from the cell culture.

39. A viral vector which contains an expression cassette according to claim 29.

40. A cell transfected with a viral vector according to claim 39.

41. A method of using the cell of claim 40 to produce a soluble , non-cleavable, chimeric HIV-1 gp160 variant comprising culturing said cell under conditions that provide for expression of said variant, and harvesting said variant from the cell culture.

42. A viral vector which contains an expression cassette according to claim 13.

43. A cell transfected with a viral vector according to claim 42.

44. A method of using the cell of claim 43 to produce a soluble , non-cleavable, chimeric HIV-1 gp160 variant comprising culturing said cell under conditions that provide for expression of said variant, and harvesting said variant from the cell culture.

L17 ANSWER 6 OF 16 USPATFULL on STN
2001:147466 Immunogenic compositions comprising soluble, non-cleavable, chimeric HIV-1 gp160-variants.
Kieny, Marie-Paule, Strasbourg, France
Transgene, S.A., Strasbourg, France (non-U.S. corporation)
US 6284248 B1 20010904
APPLICATION: US 1995-472240 19950607 (8)
PRIORITY: FR 1991-5392 19910502
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Immunogenic compositions comprising soluble, non-cleavable, chimeric HIV-1 gp160 variants capable of eliciting human immunodeficiency virus type 1 (HIV-1) gp160-specific antibodies are provided. These HIV-1 gp160 variants comprise the following regions: i) a first region derived from the gp160 of a first strain of HIV-1; ii) a second region derived from the gp160 of a second strain of HIV-1 wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, derived from the gp160 of said second strain, located at the amino terminus of the recombinant envelope.

CLM What is claimed is:
1. An immunogenic composition capable of eliciting human immunodeficiency virus type 1 (HIV-1) gp160-specific antibodies in a mammal comprising a soluble, non-cleavable, chimeric HIV-1 gp160 variant, said variant comprising the following regions: i) a first region derived from the gp160 of a first strain of HIV -1 consisting of amino acids X to Y, wherein X is a number from 1 to 271 and Y is a number from 306 to 482; ii) a second region derived from the gp160 of a second strain of HIV-1 consisting of amino acids Y+1 to the carboxyl terminus of the envelope, wherein said second region fails to contain functional major and minor proteolytic cleavage sites (amino acids 483-486 and 475-479, respectively) and functional major and minor hydrophobic domains (amino acids 487-516 and 659-680, respectively); and iii) an optional third region, present when X is greater than 1, said region derived from the gp160 of said second strain of HIV-1 and consisting of amino acids 1 to X-1, wherein the numbering scheme of X and Y is based upon the numbering scheme of the HIV-1 isolate BRU.

2. The immunogenic composition of claim 1 wherein said second strain of HIV-1 is selected from the group of HIV-1 strains consisting of BRU and MN.

3. The immunogenic composition of claim 1 wherein said first strain of HIV-1 is selected from the group of HIV-1 strains consisting of MN, ELI, RF, SF2C, and SC.

L22 ANSWER 41 OF 42 MEDLINE on STN
89184424 Document Number: 89184424. PubMed ID: 3237686. Improved antigenicity of the HIV env protein by cleavage site removal. Kieny M P; Lathe R; Riviere Y; Dott K; Schmitt D; Girard M; Montagnier L; Lecocq J. (Transgene S.A., Strasbourg, France.) PROTEIN ENGINEERING, (1988 Sep) 2 (3) 219-25. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The HIV env glycoprotein mediates virus infection and cell fusion through an interaction with the CD4 molecule present at the surface of T4+ lymphocytes. Although env presents a major antigenic target, vaccinia recombinants expressing env elicit low titres of anti-env antibody (Kieny et al., Bio/Technology, 4, 790-795, 1986). To delimit the functional domains of env and to improve the immunogenicity of the vaccinia recombinants we constructed variants expressing env proteins in which the site permitting cleavage of the gp160 precursor to yield gp120 and gp41 was removed, the gp120 and gp41 moieties separated or in which the signal sequence and hydrophobic domains were replaced by equivalents from rabies virus G. Analysis of variants revealed that the gp120 moiety is alone capable of interacting with CD4 and of provoking aggregation of T4+ lymphocytes, whereas cell-associated gp41 liberated by gp160 cleavage was essential for cell fusion. The identity of the signal and transmembrane zones however appeared unimportant. Although removal of the consensus sequence permitting cleavage of gp160 prevented syncytium formation but not aggregation of T4+ lymphocytes, significant cleavage continued to take place. Removal of a second potential cleavage site blocked gp160 cleavage. The live viruses were examined for immunogenicity: recombinant 1139 which lacks both putative cleavage sites was found to elicit a 10-fold higher antibody response in experimental animals than the parental recombinant.

L22 ANSWER 40 OF 42 MEDLINE on STN
90168946 Document Number: 90168946. PubMed ID: 2106442. Detection of primary cytotoxic T lymphocytes specific for the envelope glycoprotein of HIV-1 by deletion of the env amino-terminal signal sequence. McChesney M; Tanneau F; Regnault A; Sansonetti P; Montagnier L; Kieny M P; Riviere Y. (Departement de Medecine, Institut Pasteur, Paris, France.) EUROPEAN JOURNAL OF IMMUNOLOGY, (1990 Jan) 20 (1) 215-20. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB A heterogenous population of envelope glycoprotein-specific cytotoxic effector cells are found in the peripheral blood of individuals infected with HIV-1, and in many cases env-specific lysis is not restricted by MHC molecules and is not blocked by antibody to CD3 (Riviere, Y. et al., J. Virol. 1989, 63:2270). In order to detect env-specific cytotoxic T lymphocytes (CTL) in fresh peripheral blood mononuclear cells of HIV-1-infected donors, a mutant env gene with deletion of the amino-terminal signal sequence was inserted into vaccinia virus. This deletion of the amino-terminal signal sequence was inserted into vaccinia virus. This deletion results in synthesis of an envelope protein that is not glycosylated and not expressed at the surface of infected cells. Target cells infected with this recombinant vaccinia virus are not lysed by antibody-mediated cellular cytotoxicity, but they are recognized by secondary CTL. Comparing lysis of target cells expressing gp160 of HIV-1 and the signal peptide deletion mutant, primary env-specific CTL were detected in some individuals infected with HIV-1.

L22 ANSWER 39 OF 42 MEDLINE on STN

91082443 Document Number: 91082443. PubMed ID: 1984664. The cytoplasmic tail of HIV-1 gp160 contains regions that associate with cellular membranes. Haffar O K; Dowbenko D J; Berman P W. (Department of Developmental Biology, Genentech, Inc., South San Francisco, California 94080.) VIROLOGY, (1991 Jan) 180 (1) 439-41. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The HIV-1 envelope glycoprotein gp160 associates with cellular membranes via a discrete transmembrane domain. Unlike other retroviral envelope proteins, however, gp160 also forms a secondary association with the lipid bilayer mediated by one or more regions located in the cytoplasmic tail. We have expressed the full cytoplasmic tail sequence of gp160, as a fusion protein with the HSV-1 glycoprotein D signal sequence, transiently in a human embryonic kidney cell line. Our results show that in the absence of any defined transmembrane domain or stop transfer sequence, the protein corresponding to the cytoplasmic tail of HIV-1 gp160 formed stable interactions with cellular membranes that mediated its export to the cell surface.

L22 ANSWER 32 OF 42 MEDLINE on STN
94123987 Document Number: 94123987. PubMed ID: 8293993. The intracellular production and secretion of HIV-1 envelope protein in the methylotrophic yeast Pichia pastoris. Scorer C A; Buckholz R G; Clare J J; Romanos M A. (Wellcome Research Laboratories, Beckenham, Kent, UK.) GENE, (1993 Dec 22) 136 (1-2) 111-9. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein, gp120 (ENV), is required in large quantities for immunological studies and as a potential vaccine component. We have expressed the DNA encoding gp120 in a highly efficient expression system based on the methylotrophic yeast, Pichia pastoris. The native gene was found to contain a sequence which resembled a Saccharomyces cerevisiae polyadenylation consensus and acted as a premature polyadenylation site in P. pastoris, resulting in the production of truncated mRNA. As full-length mRNA was produced in S. cerevisiae, this indicates differences in mRNA 3'-end formation between the two yeasts. Inactivation of this site by site-directed mutagenesis revealed several additional fortuitous polyadenylation sites within the gene. We have designed and constructed a 69%-synthetic gene with increased G + C content which overcomes this transcriptional problem, giving rise to full-length mRNA. High levels of intracellular, insoluble, unglycosylated ENV were produced [1.25 mg/ml in high-density (2 x 10(10) cells per ml) fermentations]. ENV also was secreted from P. pastoris using the S. cerevisiae alpha-factor prepro secretion leader and the S. cerevisiae invertase signal sequence. However, a high proportion of the secreted product was found to be hyperglycosylated, in contrast to other foreign proteins secreted from P. pastoris. There also was substantial proteolytic degradation, but this was minimized by maintaining a low pH on induction. Insoluble, yeast-derived ENV proteins are being considered as vaccine antigens and the P. pastoris system offers an efficient method of production.

L22 ANSWER 31 OF 42 MEDLINE on STN
94378501 Document Number: 94378501. PubMed ID: 8091657. Control of expression, glycosylation, and secretion of HIV-1 gp120 by homologous and heterologous signal sequences. Li Y; Luo L; Thomas D Y; Kang C Y. (Department of Zoology, Faculty of Science, University of Western Ontario, London, Canada.) VIROLOGY, (1994 Oct) 204 (1) 266-78. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The HIV-1 gp120 signal sequence of 30 amino acids is longer than most glycoprotein signal sequences and contains an average of 5 positively charged amino acids. The HIV-1 gp120 gene with its natural signal sequence expressed in any prokaryotic or eukaryotic expression systems showed extremely low levels of synthesis and secretion. However, deletion of the HIV-1 gp120 signal sequence results in production of large quantities of a nonglycosylated form of gp120 in Spodoptera frugiperda cells. Substitution of the gp120 natural signal sequences with the signal sequences from honeybee mellitin or murine interleukin 3 promotes a high level of expression of a glycosylated form of gp120 and efficient secretion. These heterologous signal sequences contain one (mellitin) or no (IL-3) positively charged amino acids and led us to investigate the role of the positively charged amino acids in the signal sequence of HIV-1 gp120. Four charge-altered forms of the gp120 signal sequence of HIV-1 were constructed by site-directed mutagenesis in which the positively charged amino acids were sequentially substituted with neutral amino acids. The results of these experiments showed that the expression and secretion of gp120 was progressively increased by eliminating the positively charged amino acids in a stepwise fashion. However, the substitution of all positively charged amino acids resulted in the accumulation of nonglycosylated gp120 within the cells with decreased amounts of the glycosylated form of gp120. These results demonstrate that the positively charged amino acids in the signal sequence of HIV-1 gp120 are key factors in determining its poor expression and secretion. Analyses of intracellular transport and folding of gp120 further indicate that the presence of a highly charged, uncleaved signal sequence is an important factor limiting transport of gp120 from the rough ER to the Golgi apparatus.

L22 ANSWER 20 OF 42 MEDLINE on STN
97353054 Document Number: 97353054. PubMed ID: 9209312. Mutational analysis of the 5' noncoding region of human immunodeficiency virus type 1 genome. Hirota M; Koyanagi Y; An D S; Iwanaga Y; Yamamoto N; Shimotohno K. (Virology Division, National Cancer Center Research Institute, Tokyo.) LEUKEMIA, (1997 Apr) 11 Suppl 3 102-5. Journal code: 8704895. ISSN: 0887-6924. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Retrovirus particles are released by budding from the membranes of infected cells. In the course of virus production, particularly during the late stage, viral genomic RNA is incorporated specifically into virion particles. This specific incorporation of the genomic RNA requires a packaging signal sequence. A region that functions as the packaging signal was mapped to a location upstream of the gag open reading frame on the HIV-1 viral genome. In addition of this packaging signal, other cis-acting elements that are scattered throughout the genome are also required for efficient packaging. The region upstream of the splice donor site is probably important for dimer formation. Therefore, we focused on one region located between the 3' end of the primer binding site and the 5' splice donor site of HIV-1. Experiments were conducted to investigate how deletions or point mutations in this region affect both dimerization in vitro and the production of infectious virus particles. A series of RNAs of varying lengths containing the 5' noncoding region were generated, and genomic dimerization of the altered viral RNA was analyzed in vitro. One RNA construct which consisted of 112 nucleotides (nt) from nt 639 to nt 750 formed a heterodimeric complex with the RNA which consisted of 200 nucleotides from nt 551 to nt 750. We then constructed proviruses with mutations in the 639 to 750 nt region and assayed for virus production.

Several mutants that lacked the complementarity necessary to form a possible stem-loop structure in this region showed decreased production of infectious virus particles. Moreover, both deletion of this region and randomization of its nucleotide sequence completely impaired infectious virus production. Thus, the way that this region affects infectious virus production may be through its RNA secondary structure.

L22 ANSWER 13 OF 42 MEDLINE on STN
1998432894 Document Number: 98432894. PubMed ID: 9758745. Effect of promoters and signal sequences on the production of secreted HIV -1 gp120 protein in the baculovirus system. Golden A; Austen D A; van Schravendijk M R; Sullivan B J; Kawasaki E S; Osburne M S. (Procept, Inc., 840 Memorial Drive, Cambridge, Massachusetts, 02139, USA.) PROTEIN EXPRESSION AND PURIFICATION, (1998 Oct) 14 (1) 8-12. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB We compared insect cell production levels of secreted HIV-1 gp120 glycoprotein encoded by five different baculovirus expression constructs. Combinations consisting of one of two baculovirus promoters (very late or hybrid late/very late) and one of three different signal sequences [human tissue plasminogen activator (tpa), human placental alkaline phosphatase (pap), or baculovirus envelope glycoprotein (gp67)] were constructed. Production of secreted gp120 from these constructs was analyzed in two enzyme-linked immunosorbent assay formats, one detecting the total amount of secreted gp120 protein and the other measuring the level of "active" gp120 (as defined by the ability to bind to CD4). We found that for all of the constructs, approximately 50 to 90% of the secreted gp120 protein was active. Furthermore, our results indicated that expression from either promoter yielded comparable production of secreted protein, despite the fact that transcription from the hybrid promoter begins at an earlier time. By contrast, the signal sequence had a much greater effect on the levels of secreted gp120: the tpa leader yielded the highest level of secreted protein, followed by the gp67 and pap sequences. This result suggests that transcription is not a limiting factor in the production of secreted gp120, but rather that downstream processing of the protein is more critical. Furthermore, these results confirm the notion that the "optimal" signal sequence is protein dependent and that an insect-derived signal sequence is not optimal in all cases.

L22 ANSWER 6 OF 42 MEDLINE on STN
2000114042 Document Number: 20114042. PubMed ID: 10649782. Cloning trap for signal peptide sequences. Lim S P; Garzino-Demo A. (Institute of Molecular and Cell Biology, Singapore.. mcblimsp@mcbsgs1.imcb.nus.edu.sg) . BIOTECHNIQUES, (2000 Jan) 28 (1) 124-6, 128-30. Journal code: 8306785. ISSN: 0736-6205. Pub. country: United States. Language: English.

AB Novel secreted and/or type I transmembrane proteins containing N-terminal signal sequences have been successfully cloned using the signal sequence trapping (SST) method. Often this involves random cloning of short 5' cDNA terminal ends into an epitope-tagged expression vector and the detection of expressed recombinant proteins on the cell surfaces of transfected cells with an antibody to the tagged epitope. Here, we report a novel cloning system for the detection of secreted proteins also using SST. In this method, we used the human immunodeficiency virus (HIV-1) p24 as the epitope for tagging. To test the system, two constructs were created. The 5' terminal end of a human beta-chemokine (which was regulated upon activation, expressed by normal T cells and presumably secreted [RANTES])

and the 5' end of a human CD4 receptor were cloned upstream of and in-frame with the p24 cDNA. Secreted p24 was detectable in the culture media two days after transfection of either DNA construct into the human cell lines, HeLa and 293T. When the chimeric p24 expression constructs were transfected at a ratio of 1:100 to the vector pcDNA3.1(+), p24 could still be detected in cell supernatants. The use of a secreted viral antigen like HIV-1 p24 (or of any noncellular protein) as a marker in SST cloning approaches is likely to be advantageous because it reduces the background noise in detection and also renders this system suitable for high-throughput screening.

L22 ANSWER 5 OF 42 MEDLINE on STN
2000399341 Document Number: 20334807. PubMed ID: 10873786. The HIV-1 Env protein signal sequence retards its cleavage and down-regulates the glycoprotein folding. Li Y; Luo L; Thomas D Y; Kang C Y. (Siebens-Drake Research Institute, The University of Western Ontario, London, Ontario, N6G 2V4, Canada.) VIROLOGY, (2000 Jul 5) 272 (2) 417-28. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Secretory proteins and most membrane proteins are synthesized with a signal sequence that is usually cleaved from the nascent polypeptide chain, during its transport, into the lumen of the endoplasmic reticulum (ER). We have analyzed the kinetics of the cleavage of the HIV-1 Env protein signal sequence from gp160 and gp120 in HeLa, BHK, and Jurkat cells. Furthermore, we have determined the effects of this cleavage on the association of the gp160 and gp120 glycoproteins with the ER protein calnexin and the effects of the signal sequence cleavage on protein folding. The cleavage of the HIV-1 Env protein signal sequence on both gp160 and gp120 occurred very slowly in all three cell lines with a t(1/2) of 45-60 min. The core glycosylated and signal-sequence-retained forms of gp160 and gp120 associated with calnexin while the signal-sequence -cleaved forms of gp160 and gp120 had disassociated from calnexin and correctly folded as determined by their ability to associate with the CD4 cellular receptor. Further analysis of the folding state of gp160 and gp120 in nonreducing SDS-PAGE revealed that the signal-sequence-retained and calnexin-associated forms of gp160 and gp120 migrated as broad, diffuse bands, whereas the signal-sequence-cleaved or CD4-associated forms of gp160 and gp120 migrated as single sharper bands. The cause of this retardation in the rate of folding and intracellular transport of HIV-1 glycoproteins was localized to their signal sequences by fusing the vesicular stomatitis virus G protein with the HIV-1 Env protein signal sequence and expressing this chimeric protein in mammalian cells. The HIV-1 Env protein signal sequence on the VSV-G protein also confers a reduced rate of cleavage and slow intracellular transport and folding of the chimeric G protein. These results provide direct evidence that in vivo the HIV-1 glycoprotein signal sequence inhibits the folding of HIV-1 Env protein. Our data also suggest a direct correlation between the rate of the signal sequence cleavage and protein folding.

L22 ANSWER 4 OF 42 MEDLINE on STN
2000400466 Document Number: 20334309. PubMed ID: 10873644. Efficiency of erythropoietin's signal peptide for HIV(MN)-1 gp 120 expression. Herrera A M; Musacchio A; Fernandez J R; Duarte C A. (Division of Vaccines, Centro de Ingenieria Genetica y Biotecnologia, Cdad. Habana,

10600, Cuba.. amherrera@cigb.edu.cu) . BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2000 Jul 5) 273 (2) 557-9. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB The HIV-1 gp120 gene with natural signal sequence expressed in eukaryotic expression systems showed extremely low levels of synthesis and secretion. Several expression systems have been used to improve the secretion levels of gp 120. In mammalian cells, the efficient expression of gp120 fused to t-PA signal peptide has been previously reported. Here, the effects of t-PA and EPO signal peptides were compared as secretion sequences for expression of gp120 in COS-7 cells. The EPO's signal peptide is used for the first time as leader sequence for secretion of foreign proteins. Our results indicated that higher amounts of secreted gp 120 were obtained when vectors containing EPO signal peptide were used.
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L25 ANSWER 102 OF 102 MEDLINE on STN
88188813 Document Number: 88188813. PubMed ID: 2895830. Cellular anti-GP120 cytolytic reactivities in HIV-1 seropositive individuals. Weinhold K J; Lyerly H K; Matthews T J; Tyler D S; Ahearne P M; Stine K C; Langlois A J; Durack D T; Bolognesi D P. (Department of Surgery, Duke University Medical Center, Durham, North Carolina.) LANCET, (1988 Apr 23) 1 (8591) 902-5. Journal code: 2985213R. ISSN: 0140-6736. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Forty-one patients seropositive for human immunodeficiency virus type 1 (HIV-1) were assessed for cell-mediated cytotoxicity (CMC) against autologous target cells bearing the major envelope glycoprotein of HIV -1, gp120. Effector lymphocytes from over 85% of seropositive patients showed CMC specific for gp120-coated targets, whereas seronegative individuals had no detectable CMC. As a group, symptomless individuals had the highest levels of CMC; patients with AIDS-related complex and AIDS showed progressively diminished reactivity. The gp120-specific CMC was mediated by a population of non-T-cell effectors phenotypically resembling NK/K cells. Cytolysis was not restricted by major histocompatibility complex determinants, as shown by killing of heterologous gp120-adsorbed targets and of HIV-1-infected cell-lines. Gp120-specific CMC was highly augmented in the presence of interleukin 2, so it may be possible to develop therapeutic strategies aimed at destruction of virus-producing cell reservoirs in infected individuals through stimulation of HIV -specific host CMC.

L25 ANSWER 93 OF 102 MEDLINE on STN
90273183 Document Number: 90273183. PubMed ID: 2190315. Induction of CD4+ human cytolytic T cells specific for HIV-infected cells by a gp160 subunit vaccine. Orentas R J; Hildreth J E; Obah E; Polydefkis M; Smith G E; Clements M L; Siliciano R F. (Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.) SCIENCE, (1990 Jun 8) 248 (4960) 1234-7. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Cytolytic T lymphocyte (CTL) responses were evaluated in humans immunized with recombinant human immunodeficiency virus type 1 (HIV) envelope glycoprotein gp160. Some vaccinees had gp160-specific CTLs that were shown by cloning to be CD4+. Although induced by exogenous antigen, most gp160-specific CTL clones also recognized gp160

synthesized endogenously in target cells. These clones lysed autologous CD4+ T lymphoblasts infected with HIV. Of particular interest were certain vaccine-induced clones that lysed HIV-infected cells, recognized gp160 from diverse HIV isolates, and did not participate in "innocent bystander" killing of noninfected CD4+ T cells that had bound gp120.

L25 ANSWER 89 OF 102 MEDLINE on STN
91132009 Document Number: 91132009. PubMed ID: 1704395. Characterization of a conserved T cell epitope in HIV-1 gp41 recognized by vaccine-induced human cytolytic T cells. Hammond S A; Obah E; Stanhope P; Monell C R; Strand M; Robbins F M; Bias W B; Karr R W; Koenig S; Siliciano R F. (Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205.) JOURNAL OF IMMUNOLOGY, (1991 Mar 1) 146 (5) 1470-7. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB A human CTL epitope located in a region of the HIV-1 envelope protein gp41 that is highly conserved among various HIV-1 strains was identified. This epitope was recognized by CD4+ CTL clones that were induced in seronegative humans by immunization with recombinant gp160. Fusion proteins carrying portions of the HIV-1 env gene and synthetic peptides were used to localize this epitope to amino acids 584-595 of the HIV-1 BRU env sequence. Only two positions within this epitope showed variation among North American HIV-1 isolates, and the substitutions were conservative in nature. The Lys to Arg substitution at position 593 abolished recognition, probably by interfering with the peptide-MHC interactions. This epitope was recognized in association with at least one subtype of the widely distributed human class II MHC specificity DPw4, namely DPw4.2. The relatively high frequency of this allele (27.2% among Caucasians) makes it likely that a larger fraction of the population would generate a response directed at this epitope than would be the case for epitopes recognized in the context of gene products of most other class II and class I loci. Interestingly, the closely related DP beta-chain allele types 4.1 and 2.1, which differ from 4.2 by 3 and 1 amino acids, respectively, were unable to present this gp41 peptide to DPw4.2-restricted clones. Comparison of the structure of this epitope with that of other peptides recognized in the context of DPw4.2 led to the identification of a consensus sequence for DPw4.2 binding peptides. Because the gp41 CTL epitope 584-595 identified here is highly conserved and is recognized in the context of a common DP allele, it may represent an important target region for vaccine development. Our results indicate that vaccines containing this epitope may induce in a significant fraction of those immunized CTL active against at least half of all HIV-1 strains.

L25 ANSWER 85 OF 102 MEDLINE on STN
92291497 Document Number: 92291497. PubMed ID: 1351088. Production of transmembrane and secreted forms of tumor necrosis factor (TNF)-alpha by HIV-1-specific CD4+ cytolytic T lymphocyte clones. Evidence for a TNF-alpha-independent cytolytic mechanism. Liu A Y; Miskovsky E P; Stanhope P E; Siliciano R F. (Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205.) JOURNAL OF IMMUNOLOGY, (1992 Jun 15) 148 (12) 3789-98. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Candidate AIDS vaccines consisting of recombinant forms of the HIV -1 envelope glycoprotein induce, in seronegative human volunteers, an env-specific T cell response that includes CD4+, MHC class II-restricted CTL capable of lysing HIV-1-infected

target cells. In this study, we have analyzed the production of the cytokines TNF-alpha and lymphotoxin (LT) by a set of env -specific CD4+ human CTL clones. TNF-alpha and LT are of interest because of their potential role in target cell destruction by CD4+ CTL. Our studies focused on the possibility that a cell surface form of TNF-alpha expressed by CTL after physiologic activation with target APC might participate in the cytolytic reactions mediated by these clones. We found that, upon interaction with target cells expressing env epitopes in the context of the appropriate MHC class II molecules, CD4+ CTL released TNF-alpha with kinetics that were rapid, compared with other cytokines, and that were generally similar to the kinetics of target cell destruction. LT secretion was not detected during the time course of the cytolytic reactions. A novel flow cytometric assay was used to show that physiologic activation of CD4+ CTL with target APC induced expression by the CTL of cell surface forms of TNF-alpha. Immunoprecipitations from activated, surface-iodinated CTL clones revealed two forms of surface TNF-alpha, a 26-kDa form, representing the transmembrane precursor of secreted TNF-alpha, as well as the 17-kDa secreted form bound to the cell surface. For a subset of CD4+ CTL, we found that treatment of CTL with cyclosporin A inhibited Ag-induced production of both transmembrane and secreted forms of TNF-alpha but had no effect on cytolysis. Thus, although transmembrane and secreted TNF-alpha produced by HIV-1-specific CD4+ CTL may have important effects in vivo, the rapid destruction of target APC by the set of CD4+ CTL clones described here occurs through a TNF-alpha-independent mechanism.

L28 ANSWER 32 OF 37 MEDLINE on STN

92368732 Document Number: 92368732. PubMed ID: 1380260. **Study of viral replication in HIV-1-infected CEM T-cell subclones which are reduced in their ability to form syncytia. Gruber M F; Hewlett I K; Simms T; Vujcic L; Manischewitz J; Golding H. (Division of Virology, Food and Drug Administration, Bethesda, MD 20892.) AIDS RESEARCH AND HUMAN RETROVIRUSES, (1992 Jun) 8 (6) 1139-46.** Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB The derivation of ethyl-methanesulfonate (EMS) mutagenized subclones from the CEM T-cell line has been described. These clones expressed CD4 and bound soluble gp120, however, two of the generated clones were markedly reduced in their ability to form syncytia after infection with either gp160-vaccinia vector or cell-free human immunodeficiency virus type 1 (HIV-1). Here, we asked at what stage(s) viral infection is blocked in these cells. Polymerase chain reaction (PCR) analysis revealed that at 6 and 72 h postinfection with HIV-1, cells of the syncytia-deficient clones expressed markedly reduced amounts of viral-specific DNA compared with cells of the parental line or the syncytia-positive clones. Long-term cultures revealed a marked delay in the appearance of reverse transcriptase (RT) activity in the supernatants of these subclones when compared with the parental line and viral replication did not lead to massive cell death. Syncytia formation in HIV-1-infected cultures of the syncytia-deficient subclones was enhanced by tumor necrosis factor alpha (TNF alpha) when added 24 h postinfection. In contrast, pretreatment with TNF alpha for 48 h followed by washing and infection of the cells with HIV-1 augmented syncytia formation of the syncytia-positive subclones, but not of the syncytia -negative subclones. Thus, the EMS-mutagenized subclones may provide a tool to study host cell factors required for the establishment of a productive HIV-1 infection and responsiveness to TNF alpha.

L28 ANSWER 29 OF 37 MEDLINE on STN
93090464 Document Number: 93090464. PubMed ID: 1457194. **HIV -1-induced cytopathogenicity in cell culture despite very decreased amounts of fusion-competent viral glycoprotein. Bosch V; Pfeiffer T. (Forschungsschwerpunkt Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, Heidelberg, Germany.) AIDS RESEARCH AND HUMAN RETROVIRUSES, (1992 Oct) 8 (10) 1815-21.** Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB In order to examine the potential role of env-induced membrane fusion in the cytopathogenic properties of HIV-1 in cell culture, the effects of mutations within the proteolytic cleavage site of gp160, which result in a reduction but not a complete absence of proteolytic processing have been further studied. Cells expressing the mutant glycoproteins were shown to be severely reduced in their capacity to form syncytia. However, viruses encoding these glycoproteins could infect cell culture cells, albeit with delayed kinetics, and, at late infection time points, resulted in complete cytolysis of the infected culture. Since amplification by polymerase chain reaction and direct sequencing of the DNA in the infected cultures confirmed the presence of the mutant and the absence of revertant DNA, this shows that the amount of fusion competent viral glycoprotein does not influence HIV-1 cytopathogenicity, but rather that other parameters must be involved in inducing cell death.

L28 ANSWER 26 OF 37 MEDLINE on STN
94031269 Document Number: 94031269. PubMed ID: 8105835. **Membrane expression of HIV envelope glycoproteins triggers apoptosis in CD4 cells. Laurent-Crawford A G; Krust B; Riviere Y; Desgranges C; Muller S; Kieny M P; Dauguet C; Hovanessian A G. (Institut Pasteur, Department of AIDS and Retroviruses, UA CNRS 1157, Paris, France.) AIDS RESEARCH AND HUMAN RETROVIRUSES, (1993 Aug) 9 (8) 761-73.** Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB The cytopathic effect of HIV-1 and HIV-2 in CD4+ lymphocytes has been shown to be associated with apoptosis or programmed cell death. Using different experimental conditions, we demonstrate here that apoptosis is triggered by cell membrane expression of the mature HIV envelope glycoproteins, gp120-gp41 complex, and their interaction with CD4 receptor molecules. Viral entry alone did not induce apoptosis but virus replication was required in order to produce the gp120-gp41 complex. Indeed, expression of the HIV env gene alone in the CD4+ T cell line (CEM) was sufficient for the induction of apoptosis. In general, syncytium formation and apoptosis induction were closely associated as both events require functional envelope glycoproteins and CD4 molecules. Nevertheless, apoptosis but not syncytium formation was suppressed by a monoclonal antibody against CD4 that does not affect gp120 binding. Furthermore, single-cell killing by apoptosis was observed in infected cell cultures treated with a monoclonal antibody against gp41, which completely abolishes the formation of syncytia. These results indicate that apoptosis is not the consequence of toxic effects induced by the formation of syncytia but is triggered by the HIV envelope glycoproteins. Therefore, cell death during HIV infection in CD4+ lymphocyte cultures is due to a specific event triggered by the gp120-gp41 heterodimer complex programming death in metabolically active cells.

L28 ANSWER 23 OF 37 MEDLINE on STN
95273813 Document Number: 95273813. PubMed ID: 7754236.
Membrane-expressed HIV envelope glycoprotein heterodimer is a powerful inducer of cell death in uninfected CD4+ target cells. Laurent-Crawford A G; Coccia E; Krust B; Hovanessian A G. (Unite de Virologie et Immunologie cellulaire, UA 1157 CNRS, Institut Pasteur, Paris.) RESEARCH IN VIROLOGY, (1995 Jan-Feb) 146 (1) 5-17. Journal code: 8907469. ISSN: 0923-2516. Pub. country: France. Language: English.

AB HIV infection of CD4+ T cells in culture results in the production of virus and induction of cell killing by apoptosis. Such a cytopathic effect is observed during infection with syncytium -inducing or non-syncytium-inducing HIV isolates. Apoptosis is triggered by the interaction of the cell membrane-expressed HIV envelope glycoprotein heterodimer gp120-gp41 complex (external and transmembrane glycoprotein complex) with the CD4 receptor. Here we demonstrate an experimental model for the induction of apoptosis independent of HIV infection, using transiently transfected HeLa cells with the HIV1 env gene as effector cells and the CD4+ MOLT4-T4 T cells as target cells. Results obtained confirm that the induction of apoptosis requires the membrane expression of the two HIV env gene products, gp120 and gp41. Single amino acid point mutations of the envelope products that affect binding to the CD4 receptor or the fusion process abrogate the capacity of the gp120-gp41 complex to induce apoptosis. Interestingly, a point mutation in the V3 loop which inhibits fusion without affecting CD4 binding also results in the abrogation of apoptosis. These observations indicate that the induction of apoptosis is an intrinsic property of the cell membrane-expressed gp120-gp41 complex, and thus should be considered as one of the functions of HIV env gene products.

L28 ANSWER 18 OF 37 MEDLINE on STN
96343993 Document Number: 96343993. PubMed ID: 8738430. **Generation of lymphocyte cell lines coexpressing CD4 and wild-type or mutant HIV type 1 glycoproteins: implications for HIV type 1 Env -induced cell lysis. Kruger U; Pfeiffer T; Bosch V. (Forschungsschwerpunkt Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, Heidelberg, Germany.) AIDS RESEARCH AND HUMAN RETROVIRUSES, (1996 Jun 10) 12 (9) 783-92.** Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB To gain more insight into the processes leading to HIV-1 Env-induced cell death, we aim to coexpress stably wild-type and relevant mutant variants of both HIV-1 Env and human CD4 in lymphocyte cell lines. Here we report on the generation and characterization of several cell lines inducibly or constitutively expressing wild-type or cleavage-defective HIV-1 glycoproteins and human CD4 either singly or in combination. Coexpression of CD4 and wild-type Env led to the formation of multinucleated syncytia, to growth arrest and cell death, effects that all could be prevented by cultivation in the presence of monoclonal antibodies that inhibit cell surface membrane fusion. **Cell lines coexpressing CD4 and mutated, noncleavable Env, detectable at the cell surface and still retaining CD4-binding capacity, were not retarded in their growth and cytolysis did not occur.** These results indicate that cell lysis requires cell surface interaction of CD4 and

gp120/41 and cleavage of gp160 to gp120 and gp41.

L35 ANSWER 33 OF 101 MEDLINE on STN
1999292907 Document Number: 99292907. PubMed ID: 10364363. **Role of the membrane-proximal domain in the initial stages of human immunodeficiency virus type 1 envelope glycoprotein-mediated membrane fusion. Munoz-Barroso I; Salzwedel K; Hunter E; Blumenthal R. (Laboratory of Experimental and Computational Biology, Division of Basic Sciences, National Cancer Institute, National Institutes of Health, Frederick, Maryland, USA.) JOURNAL OF VIROLOGY, (1999 Jul) 73 (7) 6089-92.** Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have examined mutations in the ectodomain of the human immunodeficiency virus type 1 transmembrane glycoprotein gp41 within a region immediately adjacent to the membrane-spanning domain for their effect on the outcome of the fusion cascade. Using the recently developed three-color assay (I. Munoz-Barroso, S. Durell, K. Sakaguchi, E. Appella, and R. Blumenthal, J. Cell Biol. 140:315-323, 1998), we have assessed the ability of the mutant gp41s to transfer lipid and small solutes from susceptible target cells to the gp120-gp41-expressing cells. The results were compared with the syncytium-inducing capabilities of these gp41 mutants. Two mutant proteins were incapable of mediating both dye transfer and syncytium formation. Two mutant proteins mediated dye transfer but were less effective at inducing syncytium formation than was wild-type gp41. The most interesting mutant proteins were those that were not capable of inducing syncytium formation but still mediated dye transfer, indicating that the fusion cascade was blocked beyond the stage of small fusion pore formation. Fusion mediated by the mutant gp41s was inhibited by the peptides DP178 and C34.

L36 ANSWER 13 OF 71 MEDLINE on STN
1999260653 Document Number: 99260653. PubMed ID: 10331810. **A signal sequence trap based on a constitutively active cytokine receptor. Kojima T; Kitamura T. (Department of Hematopoietic Factors, The Institute of Medical Science, University of Tokyo, Japan.) NATURE BIOTECHNOLOGY, (1999 May) 17 (5) 487-90.** Journal code: 9604648. ISSN: 1087-0156. Pub. country: United States. Language: English.

AB Targeting of secreted and cell-surface proteins to the cell membrane is mediated by a short hydrophobic stretch of amino acids, termed the signal sequence. We have developed a method that detects signal sequences in cDNA fragments based on their ability to redirect a constitutively active mutant of a cytokine receptor to the cell surface, thereby permitting interleukin-3 (IL-3)-independent growth of Ba/F3 cells. Retrovirus-mediated expression of the fusions in IL-3-dependent cells was followed by selection of clones for growth in the absence of IL-3. Infection of cells with 5x10(6) viral particles in a pilot experiment led to the isolation of 150 known and 48 novel cDNA clones, and all the known cDNA clones were found to encode secreted and cell-surface proteins. In addition, we isolated type II membrane proteins, which have not been detected by existing signal sequence trap strategies.

L36 ANSWER 49 OF 71 MEDLINE on STN
91082443 Document Number: 91082443. PubMed ID: 1984664. The cytoplasmic tail of HIV-1 gp160 contains regions that associate with cellular membranes. Haffar O K; Dowbenko D J; Berman P W. (Department of

Developmental Biology, Genentech, Inc., South San Francisco, California 94080.) VIROLOGY, (1991 Jan) 180 (1) 439-41. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The HIV-1 envelope glycoprotein gp160 associates with cellular membranes via a discrete transmembrane domain. Unlike other retroviral envelope proteins, however, gp160 also forms a secondary association with the lipid bilayer mediated by one or more regions located in the cytoplasmic tail. We have expressed the full cytoplasmic tail sequence of gp160, as a fusion protein with the HSV-1 glycoprotein D signal sequence, transiently in a human embryonic kidney cell line. Our results show that in the absence of any defined transmembrane domain or stop transfer sequence, the protein corresponding to the cytoplasmic tail of HIV-1 gp160 formed stable interactions with cellular membranes that mediated its export to the cell surface.

L36 ANSWER 58 OF 71 MEDLINE on STN
89348030 Document Number: 89348030. PubMed ID: 2669325. Computer analysis suggests a role for signal sequences in processing polyproteins of enveloped RNA viruses and as a mechanism of viral fusion. Fazakerley J K; Ross A M. (Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia 19104.) VIRUS GENES, (1989 May) 2 (3) 223-39. Journal code: 8803967. ISSN: 0920-8569. Pub. country: United States. Language: English.

AB We have used a computer program to scan the entire sequence of viral polyproteins for eucaryotic signal sequences. The method is based on that of von Heijne (1). The program calculates a score for each residue in a polyprotein. The score indicates the resemblance of each residue to that at the cleavage site of a typical N-terminal eucaryotic signal sequence. The program correctly predicts the known N-terminal signal sequence cleavage sites of several cellular and viral proteins. The analysis demonstrates that the polyproteins of enveloped RNA viruses--including the alphaviruses, flaviviruses, and bunyaviruses--contain several internal signal-sequence-like regions. The predicted cleavage site in these internal sequences are often known cleavage sites for processing of the polyprotein and are amongst the highest scoring residues with this algorithm. These results indicate a role for the cellular enzyme signal peptidase in the processing of several viral polyproteins. Not all high-scoring residues are sites of cleavage, suggesting a difference between N-terminal and internal signal sequences. This may reflect the secondary structure of the latter. Signal sequences were also found at the N-termini of the fusion proteins of the paramyxoviruses and the retroviruses. This suggests a mechanism of viral fusion analogous to that by which proteins are translocated through the membranes of the endoplasmic reticulum at synthesis.

L36 ANSWER 59 OF 71 MEDLINE on STN
89184424 Document Number: 89184424. PubMed ID: 3237686. Improved antigenicity of the HIV env protein by cleavage site removal. Kieny M P; Lathe R; Riviere Y; Dott K; Schmitt D; Girard M; Montagnier L; Lecocq J. (Transgene S.A., Strasbourg, France.) PROTEIN ENGINEERING, (1988 Sep) 2 (3) 219-25. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The HIV env glycoprotein mediates virus infection and cell fusion through an interaction with the CD4 molecule present at the surface of T4+ lymphocytes. Although env presents a major antigenic target, vaccinia recombinants expressing env elicit low titres of anti-env antibody (Kieny

et al., Bio/Technology, 4, 790-795, 1986). To delimit the functional domains of env and to improve the immunogenicity of the vaccinia recombinants we constructed variants expressing env proteins in which the site permitting cleavage of the gp160 precursor to yield gp120 and gp41 was removed, the gp120 and gp41 moieties separated or in which the signal sequence and hydrophobic domains were replaced by equivalents from rabies virus G. Analysis of variants revealed that the gp120 moiety is alone capable of interacting with CD4 and of provoking aggregation of T4+ lymphocytes, whereas cell-associated gp41 liberated by gp160 cleavage was essential for cell fusion. The identity of the signal and transmembrane zones however appeared unimportant. Although removal of the consensus sequence permitting cleavage of gp160 prevented syncytium formation but not aggregation of T4+ lymphocytes, significant cleavage continued to take place. Removal of a second potential cleavage site blocked gp160 cleavage. The live viruses were examined for immunogenicity: recombinant 1139 which lacks both putative cleavage sites was found to elicit a 10-fold higher antibody response in experimental animals than the parental recombinant.